

Characterization of the Key Flavor Compounds Contributing to the Lingering Cup Aroma in Sauce-Flavor Baijiu Using Sensomics and Flavoromics

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Abstract This study utilized sensomics and flavoromics approaches to identify the primary flavor compounds contributing to the lingering cup aroma of sauce-flavor Baijiu (SFB). 15 odor-active compounds were detected through gas chromatography–olfactometry–mass spectrometry (GC–O–MS) combined with odor activity value (OAV) analysis. A positive correlation was observed between the price and cup aroma durability across 30 SFB samples, leading to the identification of 17 compounds as potential markers with high variable importance in projection (VIP) values ($VIP \geq 1$). Finally, aroma recombination and omission tests demonstrated that 17 aroma compounds were crucial for the lingering cup aroma, while four higher fatty acid ethyl esters and L-lactic acid contributed to the durability by enhancing substance interactions within the system, sustaining presence of aroma compounds in the cup.

Keywords: Sauce-flavor Baijiu, lingering cup aroma, gas chromatography–olfactometry–mass spectrometry (GC–O–MS), odor activity value (OAV), variable importance in projection (VIP), aroma recombination

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1. Introduction

Sauce-flavor baijiu (SFB) is well-regarded by consumers for its unique sensory characteristics, which include refined sauce-like aroma, a smooth taste, a nuanced flavor profile, a lasting aftertaste, and a persistent cup aroma [1,2]. The lingering cup aroma refers to the phenomenon where the aroma of liquors remains in the empty cup for an extended period. This effect is attributed to the diverse range of flavor compounds present in high-quality liquor and their low rate of volatilization [3]. For SFB, the durability of cup aroma is a major factor to evaluate its quality [4].

Previous studies have revealed that the primary contributors to the cup aroma are high-boiling-point substances found in the later distillation fractions [5]. Wu et al. utilized solvent extraction with gas chromatography–mass spectrometry (GC–MS) and determined that ethyl oleate, β -phenyl ethanol, along with benzene acetic acid ethyl ester were shown as the main components of cup aroma in Zhenjiu and Moutai liquors [6]. Sun et al. detected 11 aroma-active compounds in the cup aroma of SFB using static headspace gas chromatography–mass spectrometry (HS–GC–MS), predominantly esters, aldehydes, and acids [7]. Sun’s group also suggested that 13 compounds, including ethyl 3-phenylpropanoate,

phenylethyl alcohol, sotolon, L-lactic acid, as well as D-lactic acid, could be the major contributors to the empty cup aroma [8,9]. To sum up, although these studies have explored the composition of the cup aroma in SFB, the key contributors of lingering cup aroma remain unclear.

Sensomics provides a targeted approach to comprehensively analyze flavor-active compounds, encompassing flavor extraction, screening, identification, and functional verification processes [10]. Headspace solid-phase microextraction combined with gas chromatography–mass spectrometry (HS–SPME–GC–MS) could extract and identify flavor compounds from samples, and this technique is employed in analyzing aroma compounds in Chinese Baijiu [11]. Odor-specific magnitude estimation (OSME) involves directly smelling extracted samples through gas chromatography–olfactometry (GC–O) without the need for dilution, and the aroma intensity (AI) is recorded through scoring, enabling the direct identification of aroma compounds that significantly contribute to the samples [12]. Aroma recombination technology is essential in molecular sensory science and is well recognized worldwide as a method for validating the accurate characterization of key aroma compounds using odor activity value (OAV) or gas chromatography–olfactometry (GC–O) [13]. However, these methods may result in incomplete flavor analysis. Flavoromics enables a comprehensive and impartial analysis of the maximum possible number of compounds

within a sample matrix [14]. It correlates sensory and analytical data through models developed using unsupervised statistical methods, such as orthogonal partial least squares-discriminant analysis (OPLS-DA) model, which effectively identifies flavor contributions according to variable importance in projection (VIP) values [15].

Therefore, the combination of these technologies enables the evaluation of flavor-contributing compounds in the lingering cup aroma of SFB. In addition, no studies to date have investigated the flavor compounds contribute to the lingering cup aroma of SFB using reconstitution experiments.

This study focuses on identifying the key flavor compounds contributing to the lingering cup aroma of SFB, as shown in Scheme 1. Firstly, the nitrogen blowing technique was used to expedite the volatilization of the cup aroma and obtain the lingering cup aroma sample. The flavor profile of cup aroma of SFB was characterized using the quantitative descriptive sensory analysis (QDA) method, and the active odorants in each sensory dimension were identified using GC-O-MS and OAVs. Meanwhile, flavor compounds contributing to the lingering cup aroma across 30 different grades of SFBs were detected using HS-SPME-GC-MS, and their VIP values were determined through OPLS-DA. Finally, the characteristic aroma compounds of the lingering cup aroma were corroborated by aroma recombination and omission experiments, leading to the proposal that higher fatty acid esters and L-lactic acid enhance compound interactions, thereby reducing volatilization rate and prolonging the persistence of the cup aroma.

2. Materials and Methods

Materials and chemicals

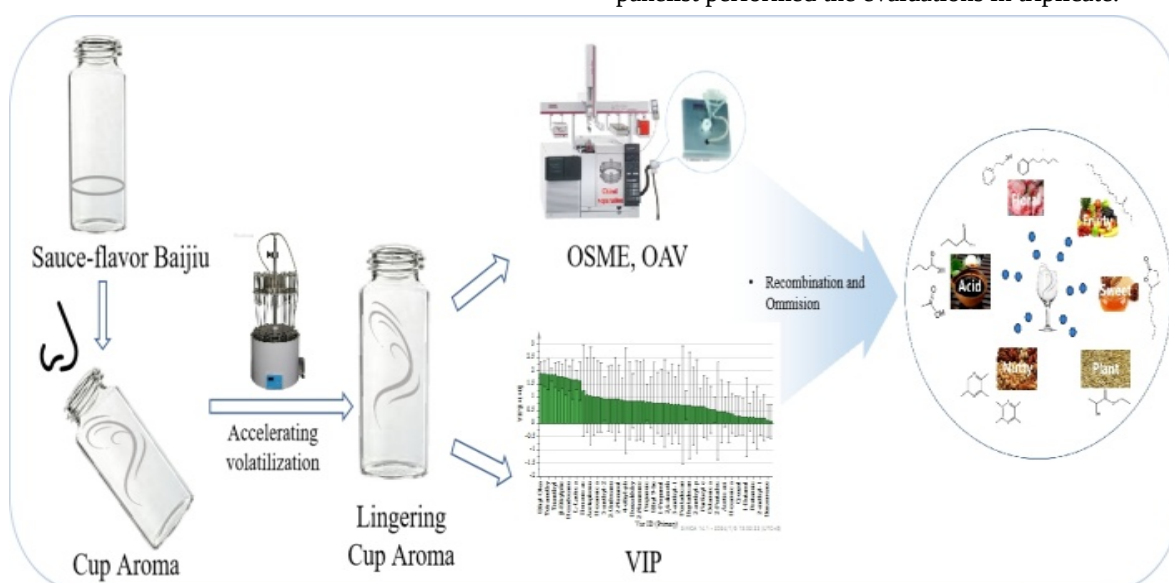
30 commercial SFB samples were purchased online, and their lingering cup aroma characteristics were evaluated by the trained panel. These 30 SFBs were divided into high (H), medium (M), and low (L) prices (H1 samples > 1500 RMB, H2 samples 1000-1500 RMB,

M1 samples 700-1000 RMB, M2 samples 500-700 RMB, L1 samples 300-500 RMB, L2 samples < 300RMB).

Ethanol (of analytical grade) was sourced from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Acetic acid ($\geq 99\%$), β -phenylethyl alcohol ($\geq 99\%$), propanoic acid ($\geq 99\%$), butanoic acid ($\geq 99\%$), 3-methylbutanoic acid ($\geq 99\%$), hexanoic acid ($\geq 99.5\%$), octanoic acid (99%), benzene acetaldehyde (98%), benzyl alcohol ($\geq 99\%$), acetic acid 2-phenylethyl ester (98%), benzene acetic acid ethyl ester (98%), trimethyl pyrazine ($\geq 99\%$), 2,3-dimethyl-5-ethylpyrazine ($\geq 99\%$), tetramethyl pyrazine ($\geq 98\%$), benzaldehyde ($\geq 99\%$), lactic acid ethyl ester (98%), γ -nonolactone (97%), decanoic acid ethyl ester (98%), tetradecanoic acid ethyl ester (98%), pentadecanoic acid ethyl ester (99%), hexadecanoic acid ethyl ester (95%), ethyl oleate (98%), linoleic acid ethyl ester (98%) were purchased from Aladdin Industrial Corporation (Shanghai, China). Benzene acetic acid (98%) was supplied by Dalian Institute of Chemical Physics, Chinese Academy of Sciences. Ultrapure water was sourced from Milli-Q IQ 7000 water purification system (Merck, Germany).

QDA analysis

QDA analysis is a combination of qualitative and quantitative sensory descriptive method, providing a comprehensive sensory profile of samples [16,17]. The QDA analysis of the cup aroma was conducted as described in reference [18]. Briefly, a trained panel of 15 members, aged between 20 and 30 years, was recruited. The panel consisted of individuals with experience in QDA analysis and long-term exposure to SFB. Each panelist signed an informed consent form and underwent training according to the specified method [19] prior to the sensory evaluation. Each panelist outlined the sensory characteristics of the cup aroma, followed by a discussion until a consensus on the descriptors was reached. Based on these discussions, nine aroma attributes were chosen to assess the cup aroma characteristics of SFB. Furthermore, panelists then evaluated the intensity of these nine aroma attributes using a 0-5 scale: 0 (no detectable odor), 1 (barely perceptible), 2 (weak), 3 (moderate), 4 (strong), and 5 (intense). Each panelist performed the evaluations in triplicate.



Scheme 1. Analysis process for identifying key flavor contributors to the lingering cup aroma of SFB

Preparation of lingering cup aroma from SFB

The preparation of the lingering cup aroma was conducted following the method in reference [20] with some modifications. Specifically, 2 mL of SFB was added into a headspace vial of 20 mL, left to stand for 10 min, and then poured out to capture the cup aroma. A nitrogen blowing instrument (Shanghai Amp-ou Scientific Instrument Co., LTD., China) was subsequently used to accelerate the volatilization of the cup aroma for 20 minutes to obtain the lingering cup aroma sample.

Extraction of lingering cup aroma compounds

The sample obtained from the previous section was processed using an automated headspace sampling system (MPSII Automatic SPME device, Gerstel Inc., Mülheim, Ruhr, Germany) equipped with a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (2 cm, Supelco Inc., Bellefonte, PA, USA). Briefly, the sample was equilibrated at 45°C for 5 minutes, followed by extraction at 70°C for 40 minutes. The fiber was subsequently inserted into the gas chromatograph injection port at 250°C for 5-minute desorption.

GC-MS analysis

An Agilent 7890A gas chromatograph connected to a 5975C mass detector (Agilent Technologies, USA) was employed to detect the extract. The system used a DB-FFAP column (30 m $\times$ 0.25 mm $\times$ 0.25 μm , Agilent Technologies Inc.) with helium as the carrier gas, set at a flow rate of 1 mL/min. The oven temperature program initiated at 40°C for 1 min, followed by an increase of 10°C/min to 80°C, then a rise of 3°C/min to 140°C, and a final increase of 10°C/min to 220°C, where it was held for 10 minutes. Compounds collected via HS-SPME were injected at 250°C and then underwent a 5-minute desorption. Mass spectra were obtained in electron ionization (EI) mode at 70 eV, scanning from m/z 35 to 350 amu in full scan mode. The ion source temperature was maintained at 230°C.

GC-O and OSME analysis

The aroma concentrates were analyzed using a gas chromatography (GC) instrument (Agilent Technologies, USA), which was equipped with a mass detector (Agilent Technologies, U.S.A), a sniffing port (ODP 2, Germany), and an air humidifier (Humidifier Gas, Germany), and linked to an Rtx-5 column (30 m $\times$ 0.25 mm $\times$ 0.25 μm ; Restek, Bellefonte, PA, SA). The GC oven and injector settings mirrored those from the earlier GC-MS experiment. Nitrogen served as the carrier gas, maintaining a steady flow rate of 1.0 mL/min. The column effluent was evenly divided between the sniffing port and the mass detector. Additionally, moist air and hydrogen were introduced into the sniffer port at flow rates of 40 and 30 mL/min, respectively, to quickly eliminate odors.

The intensity of the cup aroma components, as assessed by GC-O, was evaluated by the trained panel mentioned earlier. Each panelist documented the characteristics and intensities of the odors they perceived. The aroma intensity (AI) of the stimulus was rated on a five-point scale ranging from 1 (very weak) to 5 (very strong). Each panelist conducted the experiments in triplicate. Moreover, the aroma descriptor, retention time (RT), and AI were logged automatically. An aroma component was

recognized as active if more than half of the evaluators detected similar odors at the same RT. Lastly, the AI values were calculated as the average from all panelists.

Identification and quantitation of aroma compounds

The aroma compounds in the lingering cup aroma were identified by matching their RT and mass spectra against those in the NIST17 MS library, along with odor descriptions (Aroma) and authentic standards (Std). These substances were then semi-quantified using citronellol as an internal standard. Each sample was measured three times, and the average value was reported.

OAV and aroma character impact (ACI) calculations

The OAV of a compound was determined by dividing its concentration in the lingering cup aroma of sauce-aroma Baijiu by its odor threshold in the air [21]. According to Yang's research [22], ACI was calculated to assess each compound's contribution by dividing its OAV by the total OAV of all the compounds.

Aroma recombination and omission experiments

Recombination solution A was formulated to contain all compounds with $\text{OAV} \geq 1$ and $\text{VIP} \geq 1$ at their actual concentrations of aqueous ethanol (see Table S2). Omission tests were conducted to evaluate the contribution of specific compounds newly identified through OPLS-DA. Ten compounds were sequentially omitted from the Recombination solution A. Recombination solution A and the omission solutions were treated and analyzed using the methods described before.

Statistical analysis

All experiments were carried out in triplicate, with the data expressed as average values. Principal component analysis (PCA) and OPLS-DA were performed using XLSTAT 2019 (Microsoft company, USA) and SIMCA 14.1 (Umetrics company, Sweden), respectively.

3. Results and Discussion

Sensory analysis of cup aroma from SFB

As shown in Figure 1, the sensory profile of cup aroma of SFB was consisted of sauce, Qu, acid, plant, sweet, nutty, floral, fruity, and grassy aromas. Overall, the sauce, Qu, acid, plant, sweet, nutty, and floral aromas were notably stronger than fruity and grassy aromas, with sauce and Qu aromas being the most prominent. This phenomenon is attributed to the varying solubility of different flavor compounds in the matrix, resulting in differences in air-water partition coefficients [23].

To comprehensively investigate the aroma-active compounds within each aroma dimension described above, the GC-O time-intensity method was employed and the results are shown in Table 1. In total, 25 odorants were identified, among which β -phenylethyl alcohol and benzene acetic acid both with floral aromas, showing the strongest AI of 5. These were followed by lactic acid ethyl ester with a plant aroma, trimethyl pyrazine and tetramethyl pyrazine with a nutty aroma, acetic acid and 3-methylbutanoic acid with an acid aroma, and γ -nonolactone with a sweet flavor aroma, all of which had an AI of 4. According to these results, these compounds may be the

primary characteristic aroma compounds of the cup aroma that could serve as observation indices for optimizing nitrogen blowing treatments.

It is worth noting that eight compounds with strong

floral, fruity, sweet, smoky, herbal, and sauce aroma were not identified by GC-O-MS. Specifically, a segment of aroma with an obvious sauce flavor appeared after 40 minutes, suggesting that the contributors to the sauce flavor may have low volatility and low odor thresholds.

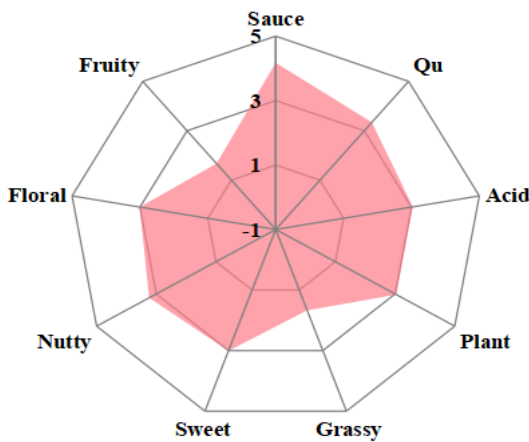


Figure 1. Sensory profile of cup aroma of SFB

Table 1. Active aromas in the cup aroma of SFB identified by GC-O-MS

RT	Compounds	Odor characteristics	Aroma dimension	Identification	AI
3.768	3-methylbutyral	Almond	Nutty	MS, Aroma, Std	1
6.051	3-methyl-1-butanol	Burnt	Nutty	MS, Aroma, Std	1
11.48	Lactic acid ethyl ester	Mushroom, plant	Plant	MS, Aroma, Std	4
12.8	2-ethyl-6-methyl-pyrazine	Nutty	Nutty	MS, Aroma, Std	1
13.375	Trimethyl pyrazine	Baked potato	Nutty	MS, Aroma, Std	4
14.21	Acetic acid	Acid	Acid	MS, Aroma, Std	4
14.928	2,3-Dimethyl-5-ethylpyrazine	Nutty	Nutty	MS, Aroma, Std	3
17.257	Tetramethyl pyrazine	Baking, nutty	Nutty	MS, Aroma, Std	4
18.904	2,3,5-Trimethyl-6-ethylpyrazin	Nutty	Nutty	MS, Aroma, Std	1
20.275	Benzaldehyde	Almond	Nutty	MS, Aroma, Std	3
20.304	Propanoic acid	Acid	Acid	MS, Aroma, Std	2
21.692	Butanoic acid	Acid	Acid	MS, Aroma, Std	3
22.116	Benzene acetaldehyde	Floral	Floral	MS, Aroma, Std	3
25.03	unknown	Floral	Floral	--	2
25.733	3-methylbutanoic acid	Acid, cheese	Acid	MS, Aroma, Std	4
26.61	Benzene acetic acid ethyl ester	Floral	Floral	MS, Aroma, Std	3
27.168	Butanedioic acid diethyl ester	Grassy	Grassy	MS, Aroma, Std	1
27.7	unknown	Smell like dry wood	Plant	MS, Aroma, Std	2
27.8	unknown	Smoking and baking	Plant	MS, Aroma, Std	3
27.851	Decanoic acid ethyl ester	Fruity	Fruity	MS, Aroma, Std	2
27.945	Hexanoic acid	Acid	Acid	MS, Aroma, Std	1
28.098	Acetic acid 2-phenylethyl ester	Rose	Floral	MS, Aroma, Std	2
28.103	Benzenepropanoic acid ethyl ester	Floral	Floral	MS, Aroma, Std	2
28.68	Benzyl alcohol	Floral	Floral	MS, Aroma, Std	3
29.321	β -phenylethyl alcohol	Rose	Floral	MS, Aroma, Std	5
31.06	unknown	Sweet	Sweet	--	3
31.245	γ -nonolactone	Milk candy	Sweet	MS, Aroma, Std	4
31.574	9-oxo-nonanoic acid ethyl ester	Sweet	Sweet	MS, Aroma, Std	1
33.09	unknown	herbal fragrance	Plant	--	4
33.6	unknown	herbal fragrance	Plant	--	3
33.9	unknown	Smoking fragrance	Plant	--	3
38.538	Benzene acetic acid	Floral	Floral	MS, Aroma	5
40.05	unknown	Sauce	Sauce	--	4

Table 2. Contents, thresholds, OAVs and ACIs of flavor components in the lingering cup aroma of SFB

Compounds	Aroma dimension	Concentration (mg/L)	Threshold (mg/L in the air)	OA V	ACI (22)
Butanoic acid	Acid	5.67	0.02	283.50	26.05%
3-methylbutanoic acid	Acid	3.2	0.014	228.57	21.00%
Benzeneacetic acid	Floral	0.54	0.003	180.00	16.54%
β -phenylethyl alcohol	Floral	5.6	0.035	160.00	14.70%
Acetic acid	Acid	60.95	0.48	126.98	11.67%
Benzeneacetaldehyde	Floral	0.27	0.004	67.50	6.20%
Trimethylpyrazine	Nutty	1.3	0.19	6.84	0.63%
Benzeneacetic acid ethyl ester	Floral	3.68	0.65	5.66	0.52%
Benzyl Alcohol	Floral	0.56	0.1	5.60	0.51%
Dodecanoic acid ethyl ester	Fruity	1.03	0.2	5.15	0.47%
Acetic acid 2-phenylethyl ester	Floral	4.38	0.9	4.87	0.45%
Lactic acid ethyl ester	Plant	51.12	14	3.65	0.34%
γ -nonolactone	Sweet	0.2	0.065	3.08	0.28%
Tetramethylpyrazine	Nutty	5.96	2	2.98	0.27%
2,3-Dimethyl-5-ethylpyrazine	Nutty	0.48	0.2	2.40	0.22%
Benzenepropanoic acid ethyl ester	Floral	0.12	0.13	0.92	0.08%
2-ethyl-6-methyl-pyrazine	Nutty	0.33	0.99	0.33	0.03%
Butanedioic acid diethyl ester	Grassy	6.17	20	0.31	0.03%
Propanoic acid	Acid	0.15	1	0.15	0.01%

OA V and ACI analyses of lingering cup aroma from SFB

The study indicated that only compounds with an OAV of 1 or higher were considered contributors to the aroma profile of the samples [24]. As shown in Table 2, 19 odorants were identified in the lingering cup aroma sample, and 15 of them had OAVs ≥ 1 .

Aromatic compounds that contribute to the smooth and mellow characteristics of Baijiu primarily originate from the catabolism of amino acids (like phenylalanine, tryptophan, and tyrosine) and the degradation of lignin [25]. Seven kinds of aromatic substances contributed to the lingering cup aroma. Among these, benzene acetic acid (16.54%), β -phenylethyl alcohol (14.7%), and benzene acetaldehyde (6.2%) were the most significant contributors.

Acids contribute cheese and acid aromas, playing a crucial role in enhancing flavor and balancing the aroma of liquor [26]. Butanoic acid (26.05%), 3-methylbutanoic acid (21%) and acetic acid (11.67%) were key contributors to the lingering cup aroma. Although the butanoic acid and 3-methylbutanoic acid had lower AI values than those of benzene acetic acid and β -phenylethyl alcohol in GC-O sniffing, their ACIs were higher, consistent with the observation that the intensity of acid aroma was greater than that of the floral aroma in the sensory profile of cup aroma.

Esters are trace components with the highest content in liquor, mainly derived from the fermentation and distillation process, and they impart pleasant fruity and sweet aromas to the liquor [27]. This study notes that lactic acid ethyl ester, γ -nonolactone, and decanoic acid ethyl ester were found to contribute to the plant, sweet, and fruity aromas respectively. It should be noted that the plant aroma dimension showed a strong intensity in the lingering cup aroma sensory profile, yet the ACI contribution of lactic acid ethyl ester was only 0.34%. On the one hand, this could be due to the aroma interactions that enhance the perceived aroma of lactic acid ethyl ester. And on the other hand, further exploration is need for the aroma-contribution substances in this dimension,

including unknown compounds identified in GC-O analysis with strong smoky and traditional Chinese medicine-like aromas.

In this study, four pyrazine compounds were found present in the lingering cup aroma sample. Other than 2-ethyl-6-methylpyrazine, trimethyl pyrazine, tetramethyl pyrazine, and 2,3-Dimethyl-5-ethylpyrazine contributed pleasant baked potato and nutty aromas. Research has shown that pyrazine compounds primarily originate from microbial metabolism and the Maillard reaction during the brewing process [28]. These compounds can enhance and intensify other aroma substances in liquor, enriching its overall aroma profile.

The results show that acids and aromatic compounds significantly influence on the lingering cup aroma of SFB. Butanoic acid and 3-methylbutanoic acid contribute both fermented food and acidic aromas, while acetic acid gives a pleasant acid aroma, which explains why the cup aroma is significantly acidic but not irritating in sensory perception. It is suggested that acids are easily absorbed and adhere to the surface of glass cups, generally exhibited low thresholds [29]. Benzene acetic acid, β -phenylethyl alcohol, and benzene acetaldehyde impart a ripe rose aroma to the cup, resulting in a strong and persistent floral aroma.

Lingering cup aroma analysis of various SFBs

In Figure 2a, the cup aroma durability of SFBs was positively correlated with price, especially in the high-price products (H1 and H2), which were distinctly different from the middle- and low- price products. The cup aroma durability intensity of M1, M2, L1, and L2 samples partly overlapped, consistent with the distribution of their cup aroma characteristics (Figure 2b).

The PCA plot explained 80.41% of the variability. The lingering cup aromas of H1 and H2 samples, positioned in the lower right quadrant of the plot, showed higher intensities of sauce, Qu and nutty aromas. In contrast, the M1, M2, M3 and M4 samples showed relatively high intensities of acidic, plant, floral, sweet and grassy aromas.

These results suggest that sauce, Qu and nutty aromas contributed more to the cup aroma, aligning with the flavor profile of the cup aroma described in Section 3.1.

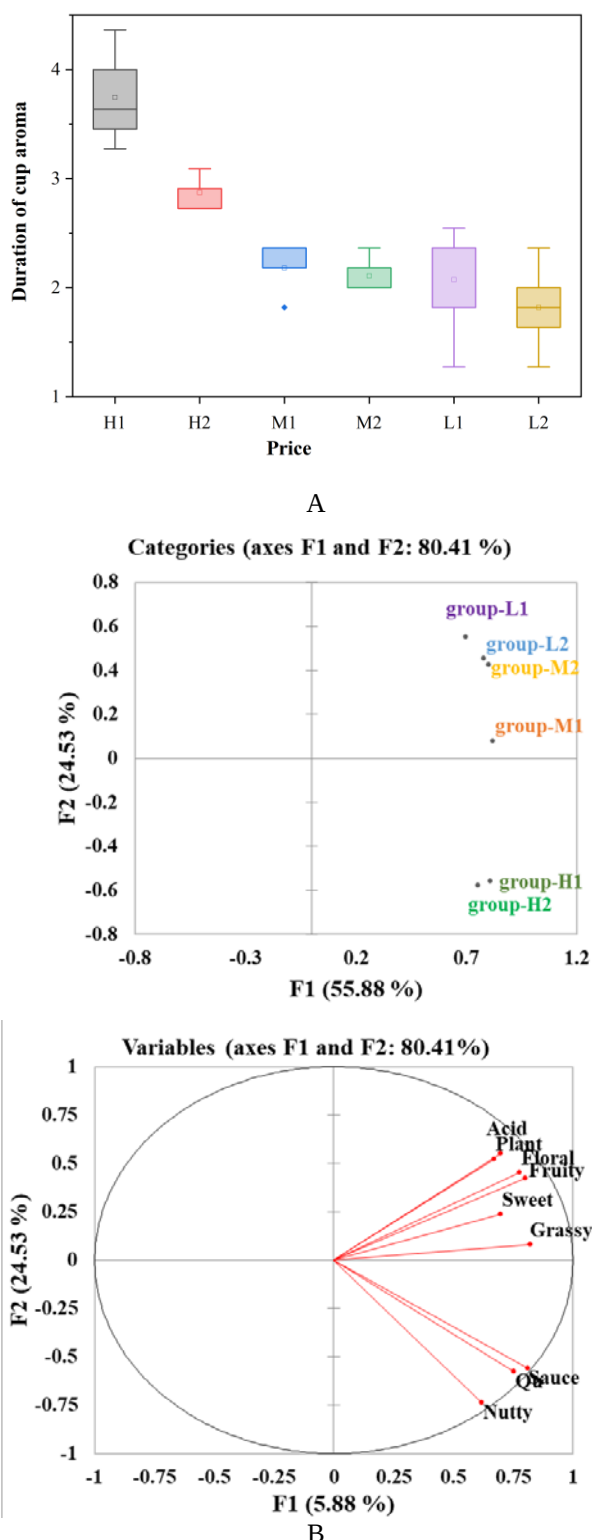


Figure 2. (a) Durability of lingering cup aroma for different prices with SFBs; (b) PCA for lingering cup aroma description

Under the optimum conditions, there are 72 compounds in the lingering cup aroma of 30 different SFB samples, including 26 esters, 16 aromatic compounds, 10 acids, 9 alcohols, 3 furans, 3 ketones, 3 pyrazines, and 2 aldehydes. The VIP value was employed to pinpoint the most potential markers of the lingering cup aroma in various SFBs.

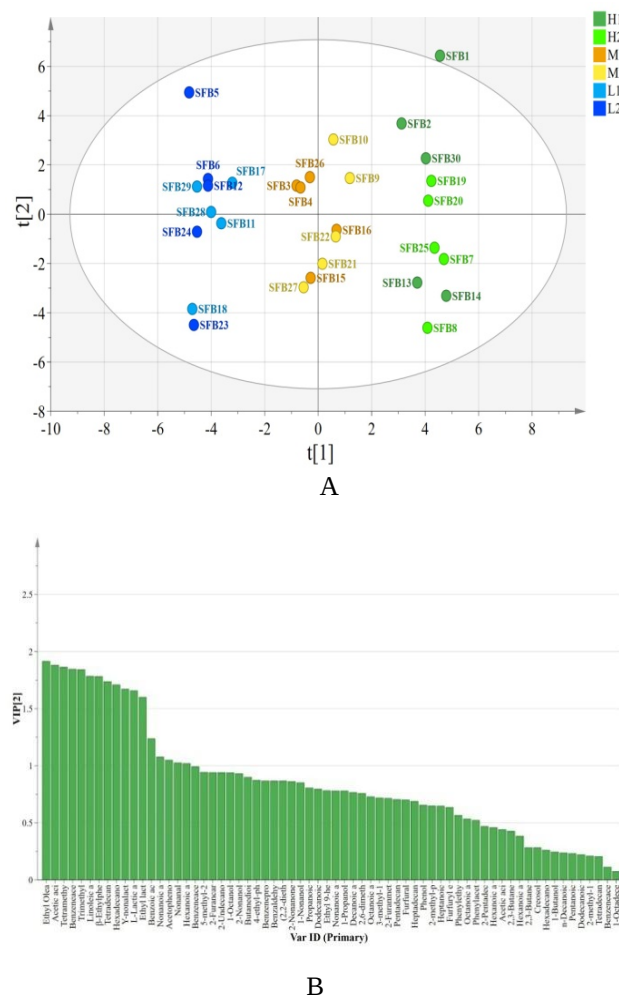


Figure 3. (a) Scores plot of flavor components influencing the lingering cup aroma in 30 SFB samples; (b) VIP plot illustrating the quantity of flavor compounds, summarizing the significance of variables in explaining the flavor compounds and their correlation with SFB samples, ranked from high to low

As shown in Figure 3a, the 30 SFBs samples were successfully differentiated into three groups, corresponding to low, medium, and high price categories from left to right. The H1 and H2 samples were positioned in the first and fourth quadrants, the M1 and M2 samples around the origin, and the L1 and L2 samples in the second and fourth quadrants. This distribution indicates that the differing composition of flavor substances composition in the lingering cup aromas of SFBs at different prices points contributes to differences in their durability and characteristics. 17 compounds with VIP >1 were identified as potential markers of the lingering cup aroma (Figure 3b), including ethyl oleate (VIP 1.92), acetic acid (1.88), tetramethylpyrazine (1.86), benzoic acid ethyl ester (1.85), trimethylpyrazine (1.84), linoleic acid ethyl ester (1.78), β -phenethyl alcohol (1.78), tetradecanoic acid ethyl ester (1.74), hexadecanoic acid ethyl ester (1.71), γ -nonolactone (1.67), L-lactic acid (1.65), Lactic acid ethyl ester (1.60), benzoic acid ethyl ester (1.24), nonanoic acid ethyl ester (1.08), acetophenone (1.05), nonanal (1.03), hexanoic acid ethyl ester (1.02). Among these, ethyl oleate, linoleic acid ethyl ester, tetradecanoic acid ethyl ester, hexadecanoic acid ethyl ester, L-lactic acid, benzoic acid ethyl ester,

nonanoic acid ethyl ester, acetophenone, nonanal, and hexanoic acid ethyl ester were newly added compared to the sensomics analysis.

Benzoic acid ethyl ester and acetophenone contribute sweet aroma and floral aroma, respectively, while nonanoic acid ethyl ester, nonanal and hexanoic acid ethyl ester impart fruity aroma. Lactic acid, a non-volatile organic acid, significantly impacts the taste and aftertaste of SFB [30]. Similar to lactic acid, higher fatty acid ethyl esters (such as ethyl oleate) have no direct contribution to the aroma but enhance the mellow sensations of SFB while serving as sustained release agents and carriers for some volatile flavor substances.

Aroma recombination and omission studies

Aroma recombination is a method to evaluate how well the estimated aroma matches the actual aroma. As shown in Figure 4, the aroma profile of recombination model A closely resembled that of the lingering cup aroma, particularly in odor attributes like acidic, grassy, sweet, nutty, floral, and fruity aromas, whereas the intensities of the sauce, Qu, and plant odor were lower in the recombination model. Similarity analysis indicated that the aroma recombination mimicked 71.6% of the comprehensive aroma profile of the lingering cup aroma, successfully identifying the key aroma compounds.

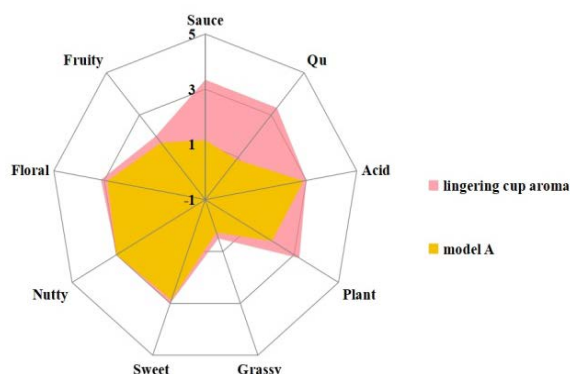


Figure 4. Aroma profiles of the lingering cup aroma in SFB in comparison with aroma recombination models (model A, recombination model)

To evaluate the aromatic contribution of the compounds recently identified through OPLS-DA, an aroma omission study was conducted to pinpoint active flavor compounds [31]. As shown in Table 3, when nonanoic acid ethyl ester and nonanal were omitted, the panel could distinguish between model A and the omission test samples. However, when benzoic acid ethyl ester, acetophenone and hexanoic acid ethyl ester were omitted, the difference from model A was not obvious. Furthermore, when ethyl oleate, linoleic acid ethyl ester, tetradecanoic acid ethyl ester, hexadecanoic acid ethyl ester, and L-lactic acid were omitted from model A, the durability of the aroma significantly decreased and the peak area responses of compounds like lactic acid ethyl ester and acetic acid in the omission model were lower than those in model A (Figure 5), which was consistent with the sensory results.

Shen et al. found that long-chain acids and their esters can enhance the intermolecular attraction of aroma compounds, resulting in the inhibition of their volatilization and a reduction in the volatilization of most

volatile compounds in liquor [32]. Sun's group found that lactic acid could lower the threshold of aroma substances such as trigonelline, enhancing their contribution to the empty cup aroma [8]. Therefore, it is speculated that the interaction of higher fatty acid ethyl esters and lactic acid with aroma compounds can inhibit the volatilization of these substances, allowing the aroma to linger in the empty cup for a longer time.

Table 3. Omission tests from recombination A model

aroma compounds omitted from the spiked sample	aroma characteristic	n/15 ^a
Benzoic acid ethyl ester	sweet	2/15
Acetophenone	Floral	4/15
Nonanoic acid ethyl ester	Fruity	11/15
Nonanal	Fruity	13/15
Hexanoic acid ethyl ester	Fruity	4/15
Ethyl Oleate		
Linoleic acid ethyl ester		
Tetradecanoic acid ethyl ester	durability of	13/15
Hexadecanoic acid ethyl ester	aroma	
L-Lactic acid		

a Number indicates a significant aroma difference between model A and omission test samples via the triangle test based on 15 panelists.

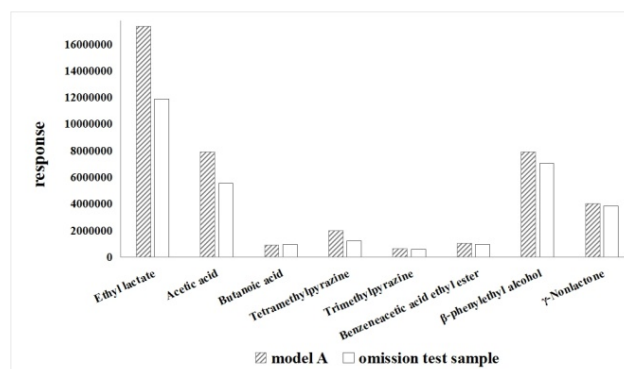


Figure 5. Peak area response of key aroma substances in model A compared with omission test sample (model A, recombination model)

4. Conclusions

In summary, the key flavor compounds responsible for the lingering cup aroma in SFB were determined through a combining of HS-SPME-GC-MS, OSME, OAV, and VIP analyses. 15 compounds with OAV >1 were acknowledged as aroma-active substances for the lingering cup aroma, and butanoic acid, 3-methylbutanoic acid, benzene acetic acid, β-phenylethyl alcohol, acetic acid, and benzene acetaldehyde were identified as the main contributors by ACI analysis. Additionally, 10 compounds with VIP >1 were further identified as the potential markers. The contributions of nonanoic acid ethyl ester and nonanal, as well as the roles of ethyl oleate, linoleic acid ethyl ester, tetradecanoic acid ethyl ester, hexadecanoic acid ethyl ester, and L-lactic acid in the durability of cup aroma, were verified through aroma recombination and omission tests. This comprehensive evaluation of the key flavor compounds in the lingering cup aroma of SFB provides theoretical insights that can serve as a reference for identifying quality batches in the SFB industry.

Ethical Guidelines

Ethics approval was not required for this research.

Author Contributions

Nian Cao: Analysis and investigation (lead); draft preparation and writing (lead). **Yubo Yang:** review and editing (lead). **Fan Yang:** Methodology (equal). **Li Wang:** Data curation (equal). All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no competing financial interests.

Data Availability Statement

Research data are available.

Supplementary methods

Preparation of aroma recombination solution

The specific recombination substances and their concentrations were shown in Table S1.

Table S1 The specific recombination substances and their concentrations

compound	Concentration (mg/L)	compound	Concentration (mg/L)
3-methylbutanoic acid ^a	49.57	2,3-Dimethyl-5-ethylpyrazine ^b	0.5
Acetic acid ^a	1444	γ-nonolactone ^b	1.72
Butanoic acid ^a	26	Benzene acetaldehyde ^b	0.35
Ethyl lactate ^a	1250.66	Acetic acid 2-phenylethyl ester ^b	0.18
Tetramethyl pyrazine ^a	19.70	Benzene acetic acid ethyl ester ^b	4.47
Trimethyl pyrazine ^a	2.06	Benzyl alcohol ^b	1.44
Tetradecanoic acid ethyl ester ^a	7.88	Dodecanoic acid ethyl ester ^b	5.3
Hexadecanoic acid ethyl ester ^a	23.61	Benzene acetic acid ^b	1.05
Ethyl oleate ^a	10.66	Benzoic acid ethyl ester ^b	0.59
Linoleic acid ethyl ester ^a	18.92	Nonanoic acid ethyl ester ^b	0.17
β-phenylethyl alcohol ^a	18.54	Acetophenone ^b	0.34
Hexanoic acid ethyl ester ^a	8.08	Nonanal ^b	11.03
Lactic acid ^c	100		

a The concentrations were quantified from the original sauce-aroma baijiu sample using a gas chromatography-flame ionization detector (GC-FID).

b The concentrations were measured from the original sauce-aroma baijiu sample using liquid-liquid microextraction combined with gas chromatography-mass spectrometry (LLME-GC-MS).

c The concentrations were measured from the original sauce-aroma baijiu sample using liquid chromatography-mass spectrometry (LC-MS).

Optimization of sample pretreatment conditions

Volume optimization of the sauce-flavor Baijiu (SFB) samples

The volume of SFB samples would affect the amount of residue on the cup wall and, consequently, the detection of flavor substances in the cup aroma. As shown in Figure S1, as the volume increased, the response of flavor compounds in the cup aroma escalated and then reached a plateau. To minimize the liquor consumption, 2 mL was selected as the optimized volume for the SFB samples.

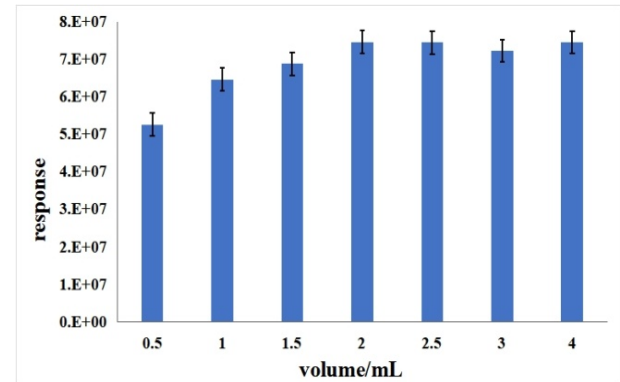


Figure S1. Influence of the volume of SFB samples on the flavor compounds response of the cup aroma

Time optimization of the nitrogen blowing treatment

The nitrogen blowing technique was employed to obtain the lingering cup aroma sample. In order to quickly obtain the lingering cup aroma sample without affecting its sensory profile or the determination of flavor substances, the duration of the nitrogen blowing treatment was optimized through sensory and instrumental analyses.

As shown in Figure S2, after 20 to 40 minutes of nitrogen blowing, important aroma dimensions such as acid, plant, sweet, nutty, and floral aromas could still be clearly perceived, whereas the sensory profile of the cup aroma changed significantly after 60 minutes. Instrumental analysis indicated that most of the volatile substances had volatilized at 20 minutes (Figure S3a) and the response of representative compounds from each aroma dimension remained high. However, when the duration was extended to 40 minutes, the response of acetic acid, γ-nonolactone and phenylethyl alcohol decreased rapidly (Figure S3b). Therefore, the optimal nitrogen blowing time was determined to be 20 minutes.

Optimization of the extraction temperature and time

According to the principles of kinetics and thermodynamics, extraction temperature and time have the most impacts on HS-SPME. Therefore, a single-factor test was adopted to study the effects of extraction temperature (50°C, 60°C, 70°C, 80°C, 90°C) and extraction time (10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes) on the flavor compound responses of the lingering cup aroma.

In Figure S4a, as extraction temperature increased from 50°C to 70°C, peak response area of the empty cup flavor substances gradually increased, indicating that higher temperature facilitate the volatilization of more aroma components. When the extraction temperature increased to

80°C, the response decreased, which might be due to the decomposition of some substances at higher temperature. Therefore, 70°C was selected as the optimal extraction temperature.

As shown in Figure S4b, the peak response area of flavor substances in the lingering cup aroma increased with the extension of extraction time up to 40 minutes and then remained relatively stable as the extraction time was further extended. Therefore, 40 minutes was selected as the optimal extraction time.

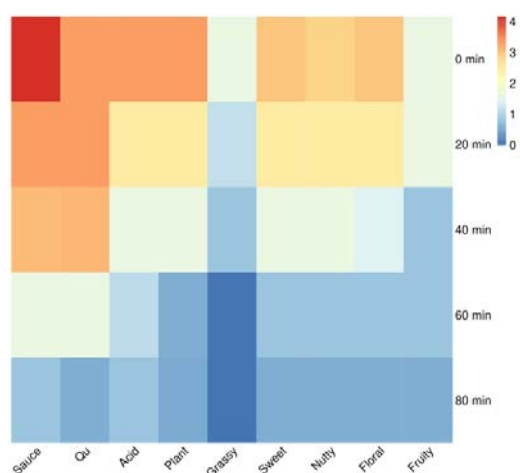
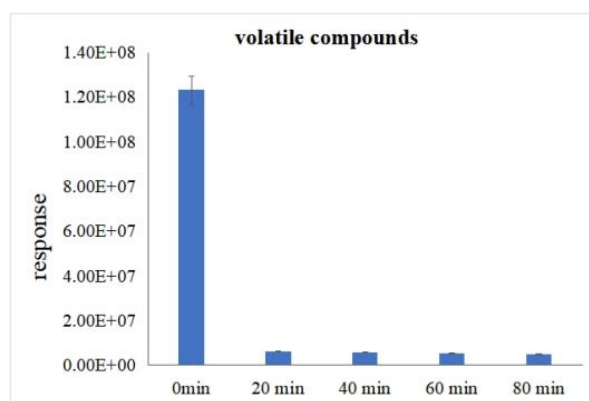
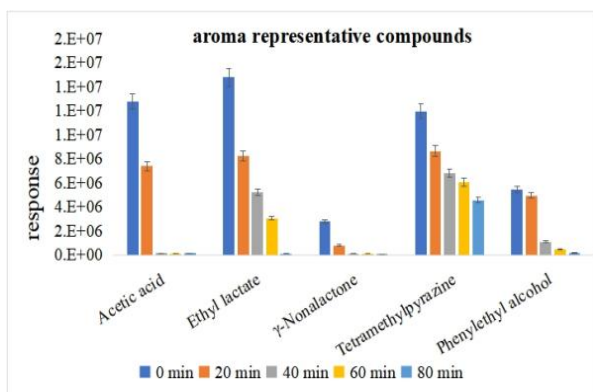


Figure S2. The sensory intensity of cup aroma varied with nitrogen blowing time

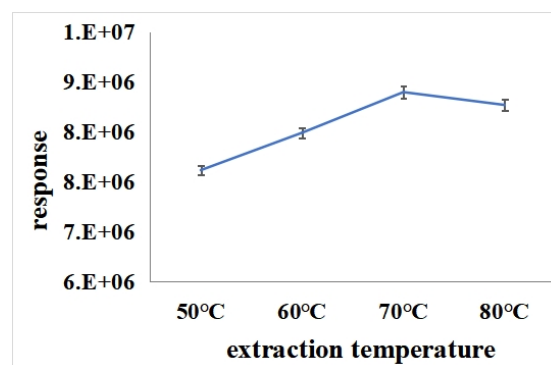


A

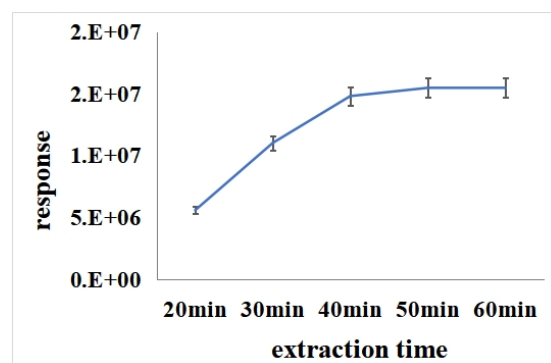


B

Figure S3. The (a) volatile compounds and (b) aroma representative compounds response of cup aroma varied with nitrogen blowing time



A



B

Figure S4. Influence of the (a) extraction temperature and (b) extraction time on the flavor compounds response of the lingering cup aroma

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